Higher fibroblast growth factor-23 increases the risk of all-cause and cardiovascular mortality in the community

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Fibroblast growth factor-23 (FGF23), a regulator of mineral metabolism, has been linked to cardiovascular disease in chronic kidney disease. As community-based data of the longitudinal association between FGF23 and cardiovascular events are lacking, we investigated a possible relationship in 727 men of the Uppsala Longitudinal Study of Adult Men population-based cohort (mean age 77 years). During a median follow-up of 9.7 years, 110 participants died of cardiovascular causes. In Cox regression models adjusted for age and established cardiovascular risk factors, higher serum FGF23 was associated with a significantly increased risk for cardiovascular mortality (hazard ratio (HR) per increased s.d. of 1.36). This relationship remained significant, albeit attenuated, after adjustment for glomerular filtration rate (GFR) (HR 1.21). FGF23 was also associated with all-cause mortality, although the association was weaker than that with cardiovascular mortality, and it was nonsignificant in fully adjusted multivariate models. Spline analysis suggested a log-linear relationship between FGF23 and outcome. Participants with a combination of high FGF23 (>60 pg/ml), low GFR (<60 ml/min), and micro-/macro-albuminuria (albumin/creatinine ratio above 3 mg/ml) had an almost eightfold increased risk compared with participants without these abnormalities. Thus, a higher FGF23 level is associated with an increased cardiovascular mortality risk in the community. Clinical trials are needed to determine whether FGF23 is a modifiable risk factor.

Kidney International (2012) **83,** 160–166; doi:10.1038/ki.2012.327; published online 5 September 2012

KEYWORDS: cardiovascular disease; chronic kidney disease; FGF-23; Klotho

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Received 10 May 2012; revised 24 June 2012; accepted 26 July 2012; published online 5 September 2012

There is growing recognition of the causal interplay between a disordered mineral metabolism and development of cardiovascular disease.[1] The clinical importance of this relationship is particularly emphasized in patients with chronic kidney disease (CKD), who suffer from a marked increased cardiovascular risk paralleled by overt changes in mineral metabolism such as hyperphosphatemia, elevated parathyroid hormone (PTH), and vitamin D deficiency.[2] However, recent studies also favor that subtle changes of these parameters, within the defined normal range for healthy individuals, are independent cardiovascular risk factors.[3–5]

Fibroblast growth factor-23 (FGF23) is a circulating hormone that has a pivotal role in the homeostatic control of mineral metabolism.[6] FGF23 reduces serum phosphate level by blocking renal phosphate uptake from the urinary filtrate and indirectly lowers serum calcium through its inhibitory action on systemic vitamin D and PTH concentrations.[7–10] FGF23 has also been suggested to be directly involved in pathological processes in the vasculature[11] and heart.[12]

FGF23 levels gradually increase in parallel with declining renal function.[13] High circulating FGF23, both in individuals with end-stage renal disease receiving dialysis treatment and in patients with moderate impairment of the glomerular filtration rate (GFR), is associated with an increased risk for multiple adverse outcomes, including mortality and CKD progression.[14–16] Yet the relationship between FGF23 and cardiovascular events in the general population has not been investigated.

We hypothesized that a higher FGF23 level, also in the concentration range not previously considered to be of pathophysiological relevance, predicts cardiovascular events. Accordingly, the aim of this study was to investigate the relationship between FGF23 and cardiovascular events in a community-based cohort of elderly men.

Table 1 | Baseline characteristics of the ULSAM cohort and by quintiles (Q1–Q5) of FGF23

Variable	All	Q1	Q2	Q3	Q4	Q5
Number of subjects	727	162	135	141	150	139
Age (years)	77.6 (0.76)	77.6 (0.66)	77.5 (0.76)	77.6 (0.80)	77.6 (0.79)	77.6 (0.82)
Serum FGF23 (pg/ml)	44 (9–162)	28 (9–33)	37 (34–40)	44 (41–47)	53 (48–59)	70 (60–162)
Glomerular filtration rate (ml/min per 1.73 m^2)	74 (17)	81 (16)	78 (14)	73 (15)	72 (13)	62 (18)
Urinary albumin/creatinine ratio (mg/μmol)	3.8 (14)	2.3 (7.4)	2.3 (4.3)	3.7 (16)	3.5 (8.6)	7.8 (25)
Body mass index (kg/m^2)	26 (3.5)	26 (3.3)	26 (3.8)	27 (3.5)	26 (3.4)	27 (3.2)
Serum total cholesterol (mg/dl)	5.4 (1.0)	5.2 (0.98)	5.5 (1.1)	5.4 (0.91)	5.4 (0.94)	5.6 (0.97)
Serum HDL cholesterol (mg/dl)	1.3 (0.32)	1.3 (0.34)	1.3 (0.31)	1.3 (0.30)	1.3 (0.33)	1.3 (0.33)
Systolic blood pressure (mm Hg)	151 (20)	148 (20)	150 (20)	151 (21)	152 (19)	151 (22)
Diabetes	97 (13%)	24 (15%)	12 (9%)	15 (11%)	27 (18%)	19 (14%)
Smoking—*n* (%)	57 (8)	10 (6)	16 (12)	14 (10)	6 (4)	11 (8)
Previous cardiovascular disease—*n* (%)	126 (27)	35 (22)	28 (21)	39 (28)	42 (28)	52 (37)
Lipid-lowering treatment—*n* (%)	125 (18)	28 (17)	21 (16)	22 (16)	23 (15)	31 (15)
Aspirin (%)	205 (28%)	34 (21%)	32 (24%)	44 (31%)	45 (30%)	50 (36%)
Antihypertensive treatment—*n* (%)	348 (48)	60 (37)	58 (43)	73 (42)	71 (47)	86 (62)
ECG-LVH	228 (31%)	44 (27%)	48 (36%)	45 (32%)	41 (27%)	50 (36%)
Serum interleukin-6 (ng/l)	3.9 (2.6)	4.0 (2.8)	3.6 (2.7)	3.6 (2.4)	3.8 (2.5)	4.2 (2.7)
Serum C-reactive protein (mg/l)	3.9 (7.0)	3.9 (7.0)	3.5 (5.8)	3.1 (3.7)	4.7 (10.4)	4.4 (6.2)
Urinary F_2 isoprostane (pmol/mmol)	0.20 (0.1)	0.21 (0.1)	0.20 (0.09)	0.19 (0.08)	0.21 (0.12)	0.19 (0.09)
Urinary 15-keto-dihydro-prostaglandin F-2α (pmol/mmol)	0.23 (0.2)	0.36 (0.20)	0.32 (0.17)	0.31 (0.15)	0.32 (0.19)	0.31 (0.19)

Abbreviations: ECG, electro cardiogram; FGF23, fibroblast growth factor-23; GFR, glomerular filtration rate; HDL, high-density lipoprotein; LVH, left ventricle hypertrophy.
Data are mean ± s.d. or median (range) for continuous variables as appropriate and *n* (%) for categorical variables.

RESULTS

Baseline characteristics for all participants and according to quintiles of FGF23 are shown in Table 1. During follow-up (median 9.7 years, range 0.3–12.9 years), 110 participants died of cardiovascular causes (incidence rate 2.07/100 person-years at risk). The incidence rates for cardiovascular and noncardiovascular mortality over FGF23 quintiles are shown in Table 2.

In our primary continuous models, higher FGF23 was associated with higher risk for cardiovascular mortality after adjustment for age and established cardiovascular risk factors (models A–B, Table 3). In multicategory and threshold models, participants in the highest quintile of FGF23 were at higher risk for cardiovascular mortality compared with participants in quintile 1 and quintiles 1–4, respectively (Model A–B, Table 3). The association between FGF23 and cardiovascular mortality was attenuated but remained statistically significant after further adjustments for GFR (model C, Table 3). In secondary models, the associations remained unaltered after the addition of albuminuria, left ventricular hypertrophy (assessed by electrocardiogram), and markers of inflammation and oxidative stress to multivariable model C (Model D, Table 3) or when replacing the hypertension treatment variable with data on specific antihypertensive medication ((angiotensin-converting enzyme inhibitors/angiotensin 2 receptor blockers, alpha-blockers, beta-blockers, calcium antagonists, or diuretics), Model B hazard ratio (HR) per s.d. increase 1.30, 95% CI 1.11–1.53, $P = 0.002$).

In crude models, FGF23 was also associated with all-cause mortality, albeit weaker than for cardiovascular mortality (Table 3). Further, after multivariate adjustment (Model C), this relationship was nonsignificant in both continuous and threshold models, and the association between FGF23 and noncardiovascular mortality was not significant in any model (data not shown). Collectively, this suggests that FGF23 primarily is associated with cardiovascular mortality and that its relationship with all-cause mortality is driven by cardiovascular deaths.

Table 2 | The association between serum FGF23 and mortality in the ULSAM cohort: incidence rates (per 1000 person-years at risk) for all-cause mortality and cardiovascular mortality

	All-cause mortality		Cardiovascular mortality	
FGF23	Number of events/numbers at risk	Incidence rates (95% CI)	Number of events/numbers at risk	Incidence rates (95% CI)
Quintile 1 (<33 pg/ml)	48/162	39 (29–52)	19/162	15 (10–24)
Quintile 2 (34–40 pg/ml)	47/136	46 (34–61)	20/136	20 (13–30)
Quintile 3 (41–47 pg/ml)	43/141	41 (31–56)	17/141	16 (10–26)
Quintile 4 (48–59 pg/ml)	50/149	45 (34–59)	21/149	19 (12–29)
Quintile 5 (⩾60 pg/ml)	64/139	70 (55–90)	33/139	36 (26–51)

Abbreviations: CI, confidence interval; FGF23, fibroblast growth factor-23.

Examination of regression splines suggested that the increase in risk is log-linear where the highest increase in HR was seen in FGF23 levels above quintile 5 (Figure 1). The cumulative incidence of cardiovascular mortality in quintile 5 vs. quintile 1–4 is shown in Figure 2.

As seen in Table 4, participants with low GFR (<60 ml/min), microalbuminuria (>3 mg/ml), and high FGF23 defined as within the highest quintile (>60 pg/ml) had a more than sixfold increase in risk for cardiovascular mortality, compared with participants with GFR >60 ml/min,

Table 3 | The association between serum FGF23, and cardiovascular and all-cause mortality in the ULSAM cohort: multivariable Cox regression

FGF23	Cardiovascular mortality				All-cause mortality			
	Model A Hazard ratio (95% CI)	Model B Hazard ratio (95% CI)	Model C Hazard ratio (95% CI)	Model D Hazard ratio (95% CI)	Model A Hazard ratio (95% CI)	Model B Hazard ratio (95% CI)	Model C Hazard ratio (95% CI)	Model D Hazard ratio (95% CI)
Continuous models								
1-s.d. increase	1.35 (1.17–1.56)***	1.36 (1.16–1.59)***	1.21 (1.02–1.45)*	1.21 (0.99–1.47)	1.21 (1.09–1.35)***	1.23 (1.10–1.39)***	1.14 (1.00–1.29)*	1.09 (0.95–1.26)
Multicategory models								
Quintile 1 (<33 pg/ml)	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent
Quintile 2 (34–40 pg/ml)	1.27 (0.68–2.39)	1.31 (0.70–2.49)	1.27 (0.67–2.71)	1.30 (0.65–2.60)	1.21 (0.81–1.81)	1.24 (0.83–1.87)	1.24 (0.82–1.86)	1.36 (0.87–2.13)
Quintile 3 (41–47 pg/ml)	1.08 (0.56–2.08)	0.99 (0.51–1.94)	0.86 (0.45–1.70)	0.73 (0.34–1.57)	1.08 (0.72–1.63)	1.07 (0.70–1.63)	0.99 (0.65–1.52)	0.99 (0.62–1.59)
Quintile 4 (48–59 pg/ml)	1.23 (0.66–2.29)	1.10 (0.59–2.06)	0.97 (0.52–1.82)	0.91 (0.47–1.78)	1.14 (0.77–1.70)	1.11 (0.74–1.65)	1.03 (0.68–1.54)	1.12 (0.72–1.73)
Quintile 5 (≥60 pg/ml)	2.42 (1.38–4.25)**	2.23 (1.25–4.00)**	1.59 (0.85–2.97)	1.56 (0.76–3.18)	1.86 (1.28–2.71)**	1.92 (1.30–2.83)***	1.55 (1.02–2.35)*	1.37 (0.85–2.16)
Threshold models								
Quintile 1–4 (<60 pg/ml)	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent
Quintile 5 (≥60 pg/ml)	2.12 (1.41–3.19)***	2.04 (1.34–3.12)***	1.59 (1.01–2.50)*	1.64 (0.96–2.80)	1.69 (1.27–2.24)***	1.75 (1.30–2.34)***	1.47 (1.08–2.01)*	1.24 (0.87–1.78)*

Abbreviations: CI, confidence interval; FGF23, fibroblast growth factor-23.
Primary models: Model A adjusted for age; Model B adjusted for age and established cardiovascular risk factors (cardiovascular disease at baseline, antihypertensive treatment, lipid-lowering treatment, current smoking, diabetes, systolic blood pressure, body mass index, total cholesterol, and high-density lipoprotein cholesterol); Model C adjusted for all covariates in Model B and glomerular filtration rate; and secondary models: Model D adjusted for all covariates in Model C and, left ventricular hypertrophy, albuminuria, and markers of inflammation and antioxidative stress.
*$P<0.05$; **$P<0.01$; ***$P<0.001$.

no albuminuria, and FGF23 < 60 pg/ml after adjustment for cardiovascular risk factors.

A statistically significant effect modification by GFR level was observed ($P=0.03$). Thus, we performed stratified analyses in participants above versus below GFR 60 ml/min. The association between FGF23 and cardiovascular mortality was higher in participants with a GFR below 60 ml/min (number of events/numbers at risk 39/146, Model C HR per s.d. increment of FGF23 1.37; 95% CI 1.02–1.85; $P=0.04$), compared with those with a GFR above 60 ml/min (number of events/numbers at risk 71/581, Model C HR per s.d. increment of FGF23 0.95; 95% CI 0.70–1.30; $P=0.76$). We found no evidence for effect modification by gender, systolic blood pressure, hypertension treatment (both as a composite variable or when investigating specific antihypertensive medication), high-density lipoprotein cholesterol, total cholesterol, lipid-lowering medication, smoking, diabetes, prevalent cardiovascular disease, body mass index, or C-reactive protein ($P>0.10$ for all).

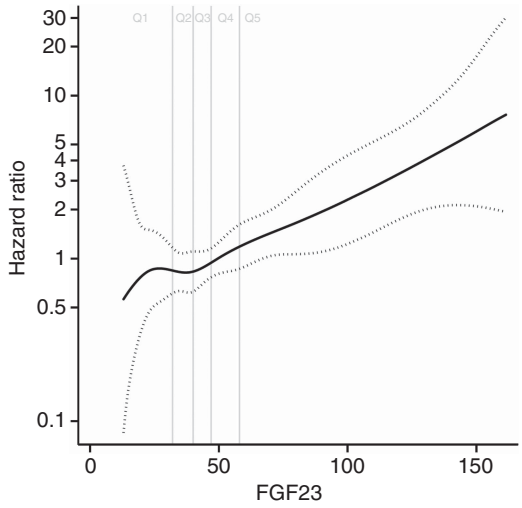

Figure 1 | Spline curve showing the relationship between fibroblast growth factor-23 (FGF23) and risk of cardiovascular mortality in the ULSAM cohort. Q, quintile.

DISCUSSION

In this study, we hypothesized that FGF23 would be associated with an increased risk for cardiovascular events in community-dwelling adults. Indeed, we confirm that higher FGF23, also in the concentration range previously not considered to be of pathophysiological relevance, is associated with increased risk for cardiovascular mortality independent of established cardiovascular risk factors. The strength of the association between FGF23 and cardiovascular mortality remained significant but was attenuated after adjustment for GFR, and stratified analysis revealed that FGF23 was not associated with cardiovascular mortality in individuals with GFR above 60 ml/min. Thus, our community-based data suggest that the potential clinical relevance of FGF23 is restricted to CKD patients. Interestingly, participants who had a combination of high FGF23, low GFR, and micro-/macro-albuminuria had substantially increased cardiovascular risk, indicating that these three different aspects of kidney pathology portray independent and additive prognostic information.

The present study corroborates with other reports proposing FGF23 as a cardiovascular risk factor. In selected

patient groups, FGF23 is associated with all-cause mortality in dialysis patients,[15,17,18] renal transplant recipients,[19] and in pre-dialysis CKD patients.[16,20,21] Further, FGF23 was independently associated with an increased risk for cardiovascular mortality in individuals free of CKD but with manifest coronary artery disease.[22] However, this is the first study to demonstrate an association between FGF23 and cardiovascular mortality in community-dwelling adults.

There are several plausible mechanisms underlying our epidemiological findings. FGF23 is a phosphaturic hormone and gradually rises in CKD to prevent the onset of hyperphosphatemia in the face of increased bone turnover and a progressive decline in functional renal mass.[23] Hence, FGF23 was proposed to be a surrogate marker of time-averaged phosphate exposure, a measure that more adequately portrays the phosphate burden and risk for development of vascular calcification compared with the measurement of serum phosphate alone.[15] FGF23 is also a potent inhibitor of vitamin D metabolism and PTH secretion, presenting the possibility that FGF23 to some extent reflects the risk exposure associated with vitamin D deficiency and hyperparathyroidism. However, the strength of FGF23 association with adverse outcomes was not attenuated by adjustments for these confounders of mineral metabolism in other studies,[15,16,19] indicating that other pathways are involved.

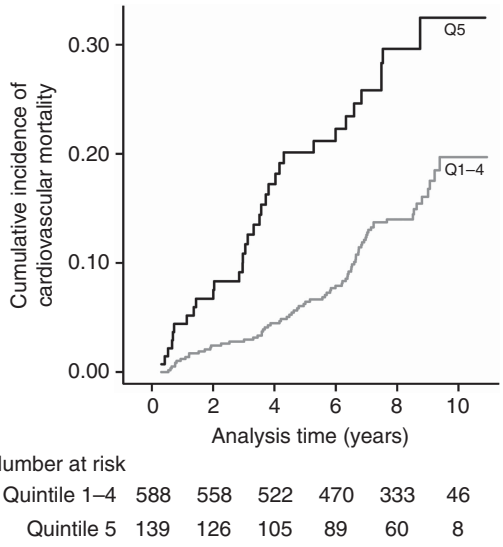

Figure 2 | Curve showing the cumulative incidence of cardiovascular mortality in the ULSAM cohort by above versus below the fifth quintile of fibroblast growth factor-23. Log-rank $P = 0.0002$. Q, quintile.

A current debate entails the possibility that the vascular system may be a target organ for FGF23, which can be assumed by the presence of the FGF23 obligatory coreceptor Klotho in vascular smooth muscle cells.[11] It remains to be elucidated whether FGF23 is a cause or consequence of vascular damage. Given that FGF23 is a mineralization inhibitor in bone,[24,25] it may exert similar effects in the vascular system, thus representing a defense mechanism against vascular calcification found in a majority of patients with advanced CKD. This hypothesis is favored by recent experimental data.[11] Additional support for vascular effects of FGF23 derives from cross-sectional studies in CKD patients and healthy individuals, in which FGF23 has been associated with dynamic measurements of vascular function,[26,27] as well as with the degree of calcification and/or atherosclerosis[28–30] in peripheral arteries, aorta, and coronary arteries.

Importantly, FGF23 was reported to directly promote cardiomyocyte growth *in vitro* and to induce left ventricular hypertrophy in mice with normal kidney function.[12] In humans, FGF23 levels have been linked to increased risk for the presence of left ventricular hypertrophy and left ventricular mass in CKD patients and in individuals free of CKD.[31–33] Although we adjusted for the presence of left ventricular hypertrophy in our multivariate models, this remains a biologically plausible pathway, especially as electrocardiogram is not an ideal tool for the determination of left ventricular hypertrophy.

The observational evidence linking FGF23 to mortality and cardiovascular disease is growing, yet it is currently unknown whether FGF23 is a modifiable risk factor. Phosphate-binding therapy, especially non-calcium-based binders, have proven effective in terms of reducing circulating FGF23 in CKD stage 3–4 when FGF23 levels are only mildly elevated compared with individuals free of CKD.[34,35] It remains elusive whether such therapy translates into a reduction in cardiovascular events, and larger observational and interventional trials would be required to define the optimal target range for FGF23. It is further possible that

Table 4 | Data portraying the interplay between the number of abnormal kidney components: FGF23, GFR, and albuminuria and the risk for cardiovascular mortality in ULSAM in Cox proportional regression models

Groups according to GFR, albuminuria, and FGF23 status	Number of events/ numbers at risk	Age-adjusted hazard ratio (95% CI)	CV risk factor–adjusted hazard ratio (95% CI)	Full model hazard ratio (95% CI)
Normal GFR, normo-albuminuria, and FGF23 < 60 pg/ml	36/375	Referent	Referent	Referent
One non-normal component	42/244	1.86 (1.19–2.91)**	1.66 (1.05–2.61)*	1.41 (0.84–2.36)
Two non-normal components	19/81	2.92 (1.67–5.10)***	2.38 (1.33–4.26)**	2.74 (1.48–5.10)**
Three non-normal components	12/28	7.96 (4.17–15.20)***	6.47 (3.28–12.74)***	5.40 (2.37–12.31)***

Abbreviations: CI, confidence interval; CV, cardiovascular; FGF23, fibroblast growth factor-23; GFR, glomerular filtration rate.
Normal GFR (⩾60 ml/min), normo-albuminuria (<3 mg/ml), and FGF23 (<60 pg/ml). CV risk factors (CV disease at baseline, antihypertensive treatment, lipid-lowering treatment, current smoking, diabetes, systolic blood pressure, body mass index, total cholesterol, and high-density lipoprotein cholesterol). The full model was adjusted for all covariates in the CV risk factor-adjusted model, left ventricular hypertrophy, and markers of inflammation and antioxidative stress.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

FGF23 becomes elevated in CKD because of the yet unknown pathological factor(s) released from the failing kidney and that such a factor would be the 'true' treatment target rather than primary reduction of FGF23 levels.

There is an ongoing debate regarding whether routine FGF23 measurements should be implemented in standard clinical care of patients with impaired kidney function as part of a risk assessment regime. FGF23 has consistently been shown to predict negative outcomes across the spectrum of CKD, and these findings are now extended to the general population with normal FGF23 levels but mild reductions in GFR. Larger clinical studies are warranted to identify relevant thresholds of FGF23 for predicting clinically meaningful outcomes.

Strengths of this study include the longitudinal study design, the detailed characterization of the study participants, and high quality of population registers in Sweden, allowing us to reach the whole population and link each individual to The National Cause of Death Register,[36] which have been shown to be in almost complete accord (99.8%) with patient journals from local hospitals.[37]

There are also some limitations to this study that needs mentioning. The cohort consists of elderly Caucasian men, which is why extrapolations of our findings to other age groups, ethnicities, and women should be done with caution. This is relevant because blacks and Hispanics have lower circulating FGF23 but higher phosphate levels than whites.[15] Similarly, estrogens are known to influence FGF23-modulating factors, including PTH, $1,25(OH)_2D$, and phosphate levels,[38,39] although a previous study did not show any gender difference for serum FGF23 in elderly men and women free of CKD.[26]

Unfortunately, we were also unable to adjust for potential confounders of mineral metabolism, including dietary intake of phosphate and vitamin D and serum parameters such as calcium, phosphate, PTH, and 25(OH)D. Previous reports support that adjustment for these variables does not alter the association between FGF23 and mortality, and some studies rather suggest a strengthened relationship when taking these variables into account.[15,16,19] Conversely, adjustment for FGF23 blunted the established association between PTH and mortality in renal transplant recipients,[19] supporting the fact that FGF23 is in the casual pathway.

Another potential confounder is aldosterone, as PTH is a regulator of both FGF23 in bone[40] and aldosterone in adrenal glands;[41] hence, adjustment for aldosterone would have been of interest to investigate whether activation of the renin–angiotensin–aldosterone system modulates the relationship between FGF23 and cardiovascular mortality. Additional studies are warranted to ultimately prove an independent association between FGF23 and cardiovascular mortality.

In summary, FGF23 is an independent predictor of cardiovascular mortality in the community-dwelling adults, supporting its potential role in the pathophysiology of cardiovascular disease.

MATERIALS AND METHODS

Study sample

The ULSAM study was initiated in 1970. All 50-year-old men, born in 1920–1924 and living in Uppsala, Sweden, were invited to a health survey, focusing at identifying cardiovascular risk factors[42] (described in detail on http://www.pubcare.uu.se/ULSAM). These analyses are based on the fourth examination cycle of ULSAM, when participants were ~77 years old (1998–2001). Of 1398 men who were invited, 838 (60%) participated. Of these, 111 were excluded owing to missing data on FGF23 ($n=77$) or covariates ($n=34$), leaving 727 participants as the present study sample. All participants gave written informed consent, and the Ethics Committee of Uppsala University approved the study protocols.

Baseline investigations

The investigations were carried out using standardized methods, including anthropometrical measurements, blood pressure, blood sampling, and questionnaires regarding socioeconomic status, medical history, smoking habits, medication, and physical activity level.[42] Venous blood samples were drawn in the morning after an overnight fast and stored at –70°C until analysis. Serum FGF23 was measured using an intact FGF23 ELISA (Kainos Laboratories, Tokyo, Japan). The intra-assay coefficient of variation was <4%. Inflammatory markers and cystatin C were measured as previously described in ULSAM.[43] GFR was calculated from serum cystatin C by using the formula $y=79.901\times \mathrm{CystC}^{-1.4389}$, which has been shown to be closely correlated with iohexol clearance.[44]

Electrographic left ventricular hypertrophy was defined as high-amplitude R waves according to the revised Minnesota code[45] together with a left ventricular strain pattern.[46] Diabetes mellitus was diagnosed as fasting plasma glucose ⩾7.0 mmol/l (⩾126 mg/dl), or the use of antidiabetic medication. Prevalent cardiovascular disease at baseline was defined as a history of ischemic heart disease or cerebrovascular disease, or Q, QS complexes, or left bundle-branch block in baseline ECG.

Follow-up and end point definitions

The Swedish Cause-of-Death register was used to define cardiovascular mortality (ICD-10 codes I00–I99). End of follow-up was 31 December 2008.

Statistical analysis

The relationship between serum FGF23 and cardiovascular mortality was investigated using Cox regression models:

(A) Age-adjusted; (B) Cardiovascular risk factor model (age, sex, systolic blood pressure, diabetes, smoking, body mass index, total cholesterol, high-density lipoprotein cholesterol, antihypertensive treatment, lipid-lowering treatment, and prevalent cardiovascular disease); (C) Cardiovascular risk factors (in Model B) and GFR.

In primary analyses, we modeled FGF23 as a continuous variable (expressed as 1-s.d. increase of serum FGF23).

We performed secondary analyses in which the following variables were added to model C: urinary albumin/creatinine ratio, electrographic left ventricular hypertrophy, C-reactive protein, interleukin-6, serum amyloid A, urinary 15-keto-dihydro-$PGF_{2\alpha}$ (reflecting COX-mediated inflammation), and urinary F_2 isoprostanes (reflecting oxidative stress) (Model D). Moreover, we replaced the antihypertensive treatment variable in the model B with the use of angiotensin-converting enzyme inihibitors/angiotensin 2 receptor blockers, alpha-blockers, beta-blockers, calcium

antagonists, or diuretics. In secondary analyses, we further used multicategory models comparing risk in FGF23 quintiles 2, 3, 4, and 5 with that in quintile 1, and threshold models (quintile 5 vs. quintile 1–4).

Tests for effect modifications by microalbuminuria, GFR, and prevalent cardiovascular disease at baseline were performed by invoking multiplicative interaction terms in Model B. To gain additional insights into potential nonlinearity of the associations, we examined the Cox regression models using penalized splines. Proportional hazards assumptions were confirmed by Schoenfeld's tests.

To evaluate the interplay between FGF23 and other aspects of kidney pathology in the risk of cardiovascular mortality, we divided the participants in the following four groups:

(1) Participants with normal GFR ($\geqslant$60 ml/min), normo-albuminuria ($<$3 mg/ml), and FGF23 $<$60 pg/ml.
(2) Participants with any one abnormality of these parameters.
(3) Participants with any two abnormalities of these parameters.
(4) Participants with all three parameters being abnormal.

A two-sided *P*-value $<$0.05 was regarded as significant in all analyses. The statistical software package STATA 10.0 (Stata Corp, College Station, TX) was used for all analyses.

DISCLOSURE

The study was investigator-initiated and -driven. The authors report no conflict of interests or connection with the industry in relation with this study.

ACKNOWLEDGMENTS

This study was supported by The Swedish Foundation for Strategic Research (TEL), Swedish Research Council (2006-6555, JÄ; K2012-55P-22137-01-06, TEL), Swedish Kidney Foundation (TEL), Swedish Heart-Lung foundation (JÄ), Karolinska Institutet (TEL), Dalarna University (JÄ), and Uppsala University (JÄ).

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